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# Male-to-Male Transmission in Extended Pedigrees With Multiple Cases of Autism

**Joachim Hallmayer, Donna Spiker, Linda Lotspeich, William M. McMahon, P. Brent Petersen, Peter Nicholas, Carmen Pingree, and Roland D. Ciaranello[1]**

*Nancy Pritzker Laboratory of Developmental and Molecular Neurobiology, and the Autism Genetics Program, Department of Psychiatry and Behavioral Sciences, Stanford University (J.H., D.S., L.L., R.D.C.), Stanford, California, Department of Psychiatry, University of Utah (W.M.M.), and Children's Behavior Therapy Unit (P.B.P., P.W., C.P.), Salt Lake City, Utah*

**Despite strong genetic influences in autism, the true mode of inheritance remains unknown. Sex differences in autism have been described in both singleton and multiplex families [Lord et al., 1982; Volkmar et al., 1993; McLennan et al., 1993; Lord, 1992]: Boys outnumber girls by 3 or 4 to 1, and so a sex-linked mode of transmission must also be considered. The key characteristic of X-linkage is that all sons of affected men are unaffected (no male-to-male transmission). In the present study, which is part of an ongoing linkage project in autism, we describe 77 multiplex autism families, 11 of who are affected cousin or half-sibling families. By using these families, it is possible to trace the path of genetic transmission and observe whether the hypothesis of X-linkage is tenable. Of 11 extended pedigrees from 77 multiplex families, six show male-to-male transmission; in these families, X-linkage can be excluded as the genetic basis for their autism. The data from the other five families are compatible with either an autosomal or an X-linked mode of transmission. The key point to emerge, then, is that autism cannot be exclusively an X-linked disorder; there must be an autosomal mode of transmission at least in some families. Thus we must consider the alternative hypotheses that autism is either entirely autosomal, or it is genetically heterogeneous, involving at least one autosomal locus with gender-specific expression, as well as a possible locus on the X-chromosome.**



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Address reprint requests to Dr. Joachim Hallmayer, Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

[1]Deceased December 15, 1994.



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## INTRODUCTION

Epidemiologic studies on autism carried out over the past two decades have consistently demonstrated a strong familiarity and a much higher prevalence in males compared to females. Studies of families of autistic probands report a sibling risk of 3–5%, which represents a 50–100 times greater risk among siblings of autistic individuals than in the normal population. This high degree of familiality has been attributed to genetic factors [for review, see Smalley et al., 1988 and Folstein and Piven, 1991]: A strong genetic component is further suggested by the high concordance rate of autism in monozygotic twins (64%) compared to dizygotic twins (9%) [Smalley et al., 1988; Folstein and Rutter, 1977; Ritvo et al., 1985; Steffenburg, 1989; Rutter et al., 1990]: Assuming a multifactorial mode of inheritance and sibling risks of 3–5%, Smalley [1991] estimated the heritability in autism to be between 80–90%, while heritability estimates based on twin concordance studies yielded estimates around 100% [LaBuda et al., 1993]:

Despite the strong genetic influences in autism, the true mode of inheritance remains unknown. Ritvo et al. [1985] proposed an autosomal recessive mode of inheritance based on surveys in multiplex families. However, their subject population was ascertained in part by advertising for multiple incidence families, and was not a population-based sample. This same group [Jorde et al., 1991] later used a systematically ascertained sample including multiplex as well as singleton cases. [Ritvo et al. 1989a] Their analysis favored a multifactorial threshold model, although a major locus model could not be rejected. However, we used the same data with the additional assumption that 11 parents from this dataset also could be diagnosed as autistic. All 11 parents showed clinical deficits in the three major areas characteristic for autism and had been given a clinical diagnosis of autism [Ritvo et al., 1988]: Changing the af-

fection status of these parents from unknown to affected suggested a dominant major single locus model (Mountain, J., Hallmayer, J., Ciaranello, R.D. and Cavalli-Sforza, L.L., unpublished observations).

The sensitivity of the presumed mode of inheritance of autism to the affected status of parents underscores a major difficulty defining the mode of transmission: the definition of the autism phenotype. The studies described above were based upon clinical diagnoses without reported reliability estimates. No standardized data on symptoms were collected, nor were standardized diagnostic instruments employed. Thus the definition of the autism phenotype can vary across studies. However, a more important problem is whether the clinically defined phenotype is a valid reflection of the underlying genotype. A number of studies have suggested that autism is the core phenotype of a broader illness, one characterized by deficits in social interaction and relatedness but not the full-blown picture of autism [Folstein and Piven, 1991; Landa et al., 1991, 1992; Piven et al., 1991]: In addition, there are several reports that siblings and parents of autistic probands show social and/or cognitive deficits. Thus evidence from several sources converges toward the point that there may be a milder, more common form of autistic disorder. It is therefore possible that parents of autistic children may themselves be mildly affected and therefore constitute the pool from which autism genes are transmitted. If true, this is an important point, because individuals with the full autistic syndrome rarely reproduce, and so the question what maintains autism gene(s) in the population remains an enigma. If criteria for this milder disorder could be established and if it could be reliably diagnosed, it might then be possible to determine a mode of transmission for autism by segregation analysis. In the absence of this, it is not possible to estimate all the necessary parameters for a segregations analysis from family studies, so determining a mode of transmission is a difficult, if not impossible task [Ott, 1990]:

Sex differences in autism have been described in both singleton and multiplex families [Lord et al., 1982; Volkmar et al., 1993; McLennan et al., 1993; Lord, 1992]: Boys outnumber girls by 3 or 4 to 1, and so a sex-linked mode of transmission must also be considered. Thus the competing hypotheses that emerge are 1) autism is due to transmission of an X-linked gene [Jones and Szatmari, 1988 [Gurling, 1986] 2) autism is due to transmission of an autosomal gene in which there is reduced penetrance in females [Ritvo et al., 1985b] 3) autism is a genetically heterogeneous disorder and both autosomal and sex-linked transmission take place, or 4) autism is a polygenic disease.

The principal formal characteristics of X-linked recessive inheritance have been well summarized by Vogel and Motulsky [1986]: The key characteristics are: 1) males are predominantly affected; 2) all the phenotypically unaffected but heterozygous daughters of affected males are carriers; 3) among the sons of heterozygous women, there will be 1:1 ratio between affected and unaffected subjects; 4) transmission occurs from affected grandfathers through healthy mothers to affected grandsons; and 5) all sons of affected men are unaffected (no male-to-male transmission). The last criterion (5) applies to X-linked dominant inheritance as well. Criteria 1 through 4 are not absolutely decisive for locating a gene on the X-chromosome because an autosomal gene with preferential expression in males could show the same pattern, but criterion 5 is a hallmark of X-linked inheritance.

The inability to define a parental autism phenotype complicates pedigree examination for X-linkage in nuclear families of affected autistic individuals. However, one way around this problem is to evaluate families with affected cousins or affected half-siblings, because it then becomes possible to trace the pathway of genetic transmission, provided the disease is sufficiently uncommon that bilineality is unlikely. In the present study, which is part of an ongoing linkage project in autism, we describe 77 multiplex autism families, 11 of which are affected cousin or half-sibling families. By using these families, it is possible to trace the path of genetic transmission and test whether the X-linkage hypothesis is tenable. The results demonstrate that X-linkage can be excluded in some but not all families, and thus the hypothesis cannot yet be rejected. In the absence of a linkage marker or disease gene, it is not possible to prove that autism is mediated by an autosomal locus with gender-dependent penetrance, and so this hypothesis is not examined here. Similarly, confirmation of genetic heterogeneity requires that at least one linked marker be found, so this hypothesis is also not addressed here.

## MATERIAL AND METHODS

### Subjects and Data Collection Procedures

Families are recruited for evaluation and inclusion in the linkage study if there are available records for at least two family members with a referral diagnosis of autism. Data collection procedures are described in Spiker et al. [1994] and are briefly summarized here. After initial referral to the linkage study, an intake telephone screening is conducted and available clinical diagnostic records are reviewed to confirm a referral diagnosis of autism in at least two of the children. Potential subjects with associated diagnoses (Fragile X, Norrie syndrome) are not enrolled in the linkage study. Written consent to participate in the study is then obtained. All children are assessed with the Autism Diagnostic Interview [ADI, LeCouteur et al., 1989], and for those children under 17 years of age, the Autism Diagnostic Observation Schedule [ADOS, Lord et al., 1989] is also administered. All assessments are videotaped, and to the greatest extent possible, interviews are conducted blinded to the status of other children in the same family. Independent evaluation of all subjects was obtained in 73% of duplex families, and approximately 30% of families with three or more autistic members. All interviewers are certified by the developers of the ADI/ADOS based on a review of an ADI practice tape yielding scoring agreement of 90% or greater.

### Scoring the ADI and Diagnostic Classifications

Scoring of the ADI is based on the guidelines prescribed by the interview's developers. To meet the ADI

ICD-10 criteria for a diagnosis of autism, the child must have a score above the prespecified cutpoint in all three symptom areas of the ICD-10 diagnostic system (social impairments, language, and communication impairments and difficulties with routines and rituals): They must also have an onset of symptoms prior to age 3 years. Individuals who meet some but not all ADI cutoffs (see below) are classed as "uncertain." Individuals for whom diagnostic information could not be obtained, either because they were not living or not available are classed as "unknown." Only those families in which two or more members meet full ADI criteria for autism ("strict multiplex") are included in the data analysis, according to criteria described in Spiker et al. [1994]:

### Reliability

Reliability of the ADI data was assessed based on independent review of 37 randomly selected ADI videotapes from the first 125 interviews conducted [Spiker et al., 1994]: The kappa coefficient for autistic vs. nonautistic was 0.90 (95% one-tailed confidence limit $>$ 0.80), the sensitivity was 0.90, and the specificity was 1.00. The kappa coefficient for not autistic versus all others was 0.90 (95% one-tailed confidence limit $>$ 0.76): The sensitivity was 0.91, and the specificity was 1.00.

## RESULTS

A total of 77 families met strict criteria for multiplex autism. The 77 families contain a total number of 221 children on whom ADI evaluations were performed. Of these, 153 (69.2%) are males and 68 (30.8%) are females; this is a highly significant deviation from a 1:1 ratio ($\chi^2 = 30.28, P < 0.001$): 168 children are autistic, 126 are males (75.0%), and 42 are females (25.0%): The ratio of autistic males to females is 3 to 1 (126/42); this is consistent with published estimates. When the autistic subjects are removed, there are 53 unaffected subjects; 27 are males and 26 are females.

Of the 77 families in the study, 11 families had either an affected cousin of affected half-sibling. Of the 52 diagnosed subjects in these 11 families, 26 are autistic, 21 are unaffected, and 5 reached the cutpoint in the ADI algorithm in one or more of the four areas (uncertain): ADI data could not be gathered from seven children because they were less than 4 years of age. The 11 extended pedigrees are shown in Figure 1.

Twenty-three males and three females were autistic. The number of autistic males was 7.67 times higher than the females, whereas among the unaffected subjects the male to female ratio was 1.1 (11/10): The overall proportion of males (72.3%) to females (27.7%) is similar to that seen in the total sample of families. In six of the eleven families (001, 002, 003, 025, 026, and 032) male to male transmission was observed, and X-linkage can therefore be excluded from this subset. In five of the families X-linkage could not be excluded (007, 015, 023, 024, and 352. In these five families, three of the males were unaffected, ten were affected, and four fell into the uncertain category. The proportion of affected males to be expected either from an X-linked or an autosomal dominant mode of transmission is 50%. The proportion observed is greater than this, but the sample size is too small for this difference to be statistically significant. The proportion of affected males to females did not differ significantly between the predicted value of 3:1 in either the entire sample of 77 families or the subset of 11 shown here $\chi^2 = 2.51$):

## DISCUSSION

To our knowledge, this is the first published report describing autism multiplex families using a standardized reliable diagnostic interview in which either cousins or half-siblings are affected with autism. These extended family structures provide an opportunity to test the possibility of X-linkage in autism.

Analysis of affected cousin and half-sibling pedigrees permits rejection of an X-linkage hypothesis when male-to-male transmission is observed. The converse of this statement is not true: absence of male-to-male transmission is consistent with but does not prove X-linkage. Of 11 extended pedigrees from 77 multiplex families, 6 show male-to-male transmission; in these families X-linkage can be excluded as the genetic basis for their autism. The data from the other five families is compatible with either an autosomal or an X-linked mode of transmission. The key point to emerge, then, is that despite the male predominance autism cannot be exclusively an X-linked disorder; there must be an autosomal mode of transmission in some families. Thus we must consider the alternative hypotheses that autism is either entirely autosomal, or it is genetically heterogeneous, involving at least one autosomal locus with gender-specific expression, as well as a possible locus on the X-chromosome.

Because the ascertainment of these families was not population based, it is not possible to provide an estimate of the fraction of families in which X-linkage can be excluded. It would be an error, for example, to conclude from these data that 55% of multiplex families transmit an autosomal locus. A precise estimate of the proportion of families in whom X-linkage can be excluded could only be obtained from a population-based survey of multiplex families, or from a sample so large as to be fairly representative of the total population of multiplex families. Overall families incompatible with X-linkage tended to be larger and had more generations over which to observe male to male transmission. This observation leads us to speculate that there is little evidence for X-linkage in these extended multiplex families.

To further test the hypothesis of an involvement of genes located on the X-chromosome in autism, we are conducting a linkage study using X-chromosomal markers in 34 families in which X-linked inheritance could not be excluded. That work is still in progress, and the results will be described upon completion of the study. Our recent work shows that autism in multiplex families is unlinked to the Fra-X locus [Hallmayer et al., 1994]: Fragile X syndrome is the most common form of genetic mental retardation, and approximately 3% of autistic individuals show the fragile X chromosome on cytogenetic testing [Piven et al., 1991]: Despite this, we found no evidence in 81 autistic subjects for either an expanded (CGG)n repeat in the FMR-1 gene or linkage of FMR-1 to autism. Taken together with this

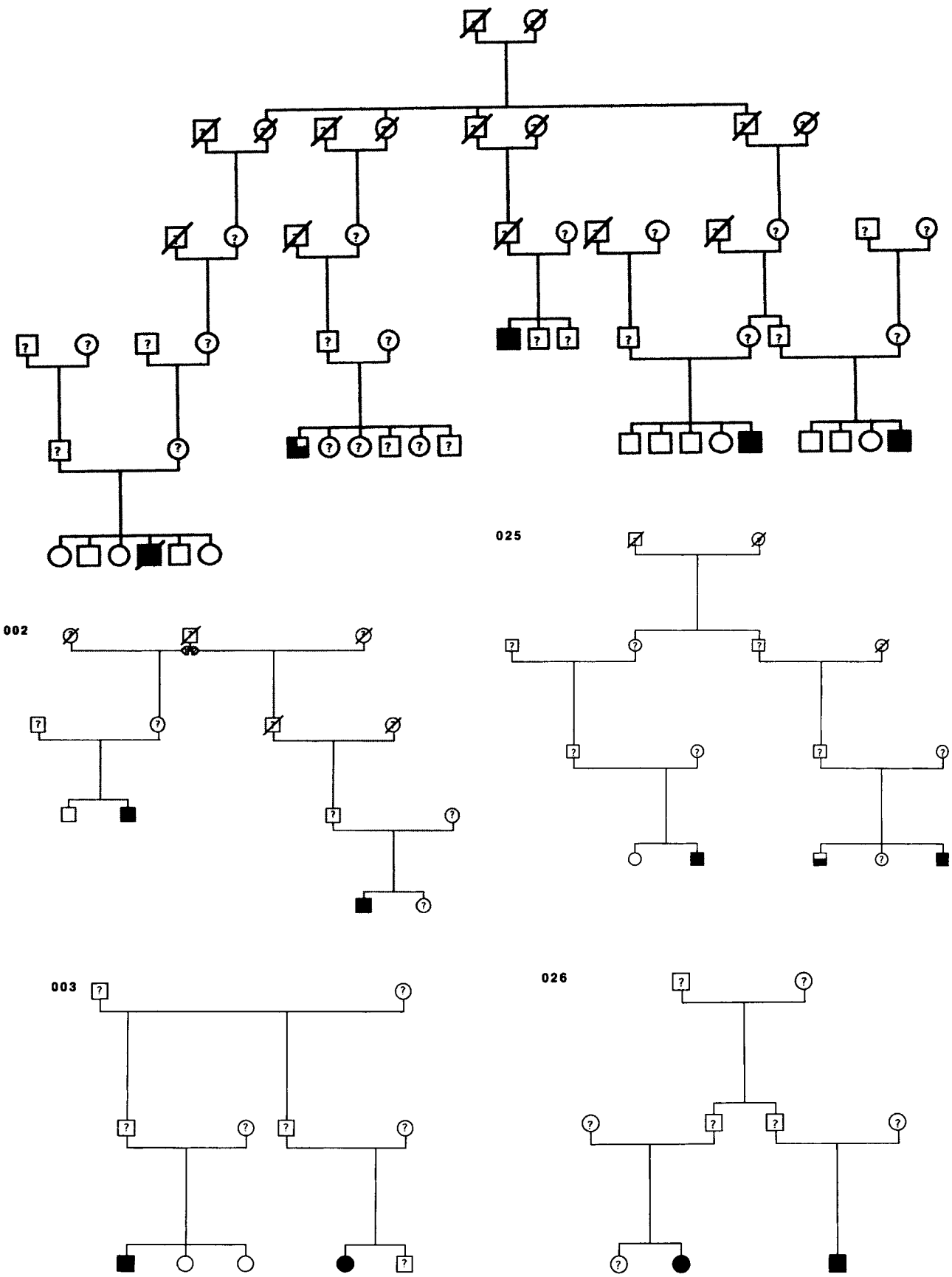


Fig. 1. Representation for 11 families in which either cousins or half-siblings are affected with autism. Completely shaded circles or squares are for autistic children; completely unshaded forms indicate not autistic, unaffected children; and partially shaded forms indicate children with an uncertain classification. The legend indicates which ADI cutpoints were met. Parents as well as children on which data could not be gathered by the ADI interview are marked with a question mark.

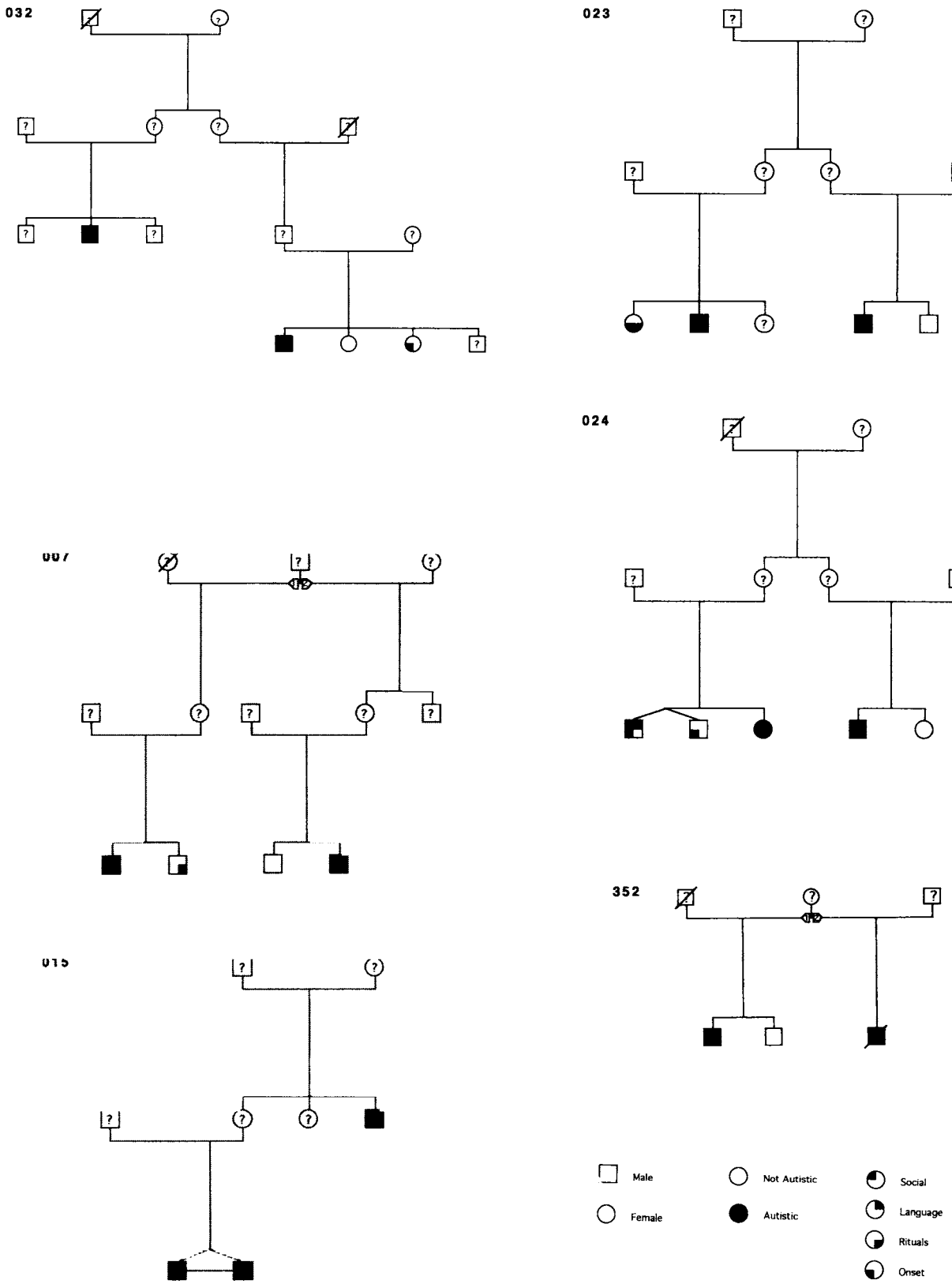


Figure 1 (continued)

report, our data suggests that if there is an autism gene on the X-chromosome, it is not linked to FMR-1, and it is present in only a subset of families.

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